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PATHOGENICITY OF A LOCAL ISOLATE OF INFECTIOUS BURSAL DISEASE VIRUS FOR EGG-TYPE CHICKENS .

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SUMMARY

Seven-week-old commercial egg-type chickens were infected with a local isolate of infectious bursal disease virus (vvIBDV) . Sacrificed and dead birds were examined at 1, 2, 3, 4, 5, 6 and 7 days post-infection (PI).

The results revealed severe clinical signs 2 days PI, the course of the disease was short with high morbidity and mortality . Sixty eight precentage of the infected birds were died , 67% of them died within 4 days PI with distinct pathological changes in the bursa of Fabricius (BF), thymus and harderian glands (GH) . These included focal lymphoid necrosis and depletion in bursal lymphoid follicles , cortical lymphoid necrosis in thymus , and plasma cell depletion in GH .

INTRODUCTION

Infectious bursal disease (IBD), is an acute viral disease of young chickens caused by a double-stranded RNA virus (IBDV) of the Birna- viridae . IBDV is well known to have a selective tropism for B-lymphocytes and cause necrosis of lymphoid follicles of the Bursa of Fabricius (BF) (Craft et al., 1990 ; Ismail et al., 1987; Sharma et al., 1989). During the last decade , outbreaks of an acute IBD with unusual high mortality occurred in Europe (Chettle et al.,1989) and then spread widely to other countries including Egypt (El-Batrawi,1990; Khafagy et al., 1990 and 1991; Ahmed , 1991 ; Sultan, 1995) . Chickens inoculated with isolates from such outbreaks (very virulent strains) developed thymic and bone marrow lesions as well as necrosis of the BF (Nakamura et al., 1992 ; Nunoyo et al., 1992 ; Tsukamoto et al., 1992) . The mechanisms by which the BF is

destroyed during infection are still unclear. Besides necrosis, apoptosis has also been observed recently after in vitro (Vasconcelos and Lam , 1994 ; Tham and Moon , 1996) and in vivo infection with IBDV (Inoue et al., 1994 ; Vasconcelos and Lam , 1995 ; Lam , 1997 ; Ojeda et al., 1997) in lymphoid bursal cells . The suppression of the immune system is principally caused by alteration of humoral response, but some investigators have shown also alteration in the cell - mediated immune response following IBDV infection (Craft et al., 1990 ; Sharma and lee , 1983) .

The objective of this study was to determine the pathogenicity of a local isolate of IBDV for egg-type chickens ,with special reference to its effects on central and peripheral organs of the immune system .

MATERIALS AND METHODS

Experimental chickens: Two hundred , one-day-old commercial white egg-type (LSL) male chicks from a commercial hatchery, which possessed maternally derived antibodies (MDA) against IBDV, were used .

Reference antigen and antiserum: Known positive and negative IBDV antigen and antiserum were obtained from Divinders Institute , The Netherlands , for use in agar gel pricipitation test (AGPT) .

IBD-virus: A local IBDV strain , isolated and identified by Sultan (1995), was used for experimental infection in the form of infected bursal homogenate given intra-ocularly in a dose of 100 µl / bird . This dose causes 70-80% mortality in susceptible egg-type chickens .

Agar gel precipitation test (AGPT): The test was used to demonstrate seroconversion to IBDV and to detect IBDV antigen(s) in BF of infected chickens . The agar medium was prepared as described by Wood et al. (1979) .

Enzyme-linked-immunosorbent assay (ELISA): The test was prallelly used to demonstrate seroconversion to IBDV in infected chickens , using commercial ELISA kits supplied by IDEXX Laboratories , Inc., Westbrook, ME 04092 . Application and interpretation of the test were undertaken according to the instructions of the kits producer .

Histopathological examination: BF, thymus and GH were collected from five infected and non-infected control chickens at 1, 2, 3, 4, 5, 6 and 7 days PI and specimens were fixed in 10% neutral formalin and processed in the usual way for paraffin sections which were stained by hematoxylin and eosin .

IBDV antigen detection: Bursae from infected dead birds and those which survived the infection were homogenized, diluted 1:1 in phosphate buffer saline (PH 7.2) and individually examined with the AGPT against the reference IBD antiserum according to Wood et al.(1979).

Experimental design: Waining of maternally derived antibodies (MDA) from the experimental chicks was followed up weekly starting

from 1 day up to 49 days of age . For this purpose 10 random blood samples were collected at different intervals and sera were examined individually by AGPT and ELISA .

At day 49 of age , a group of 100 birds were subjected to oculonasal challenge with 100 µl / bird of IBDV local isolate and observed for 7 days PI, and the rest of the experimental birds were kept without challenge as control group . Clinical signs , mortality pattern and rate , and gross lesions were recorded and IBDV antigen detection in bursal homogenates of dead and survivor birds was carried out . In addition , bursa : body weight index and bursal index were determined respectively for infected and control birds after Lucio and Hitchner (1979 and 1980) and Sharma et al. (1989). Seroconversion was also followed in these birds up to 7 days PI by AGPT .

Furthermore, histopathological examination of BF, thymus and GH of five dead and / or sacrificed birds was carried out at 1- day intervals during the observation period . The severity of lymphoid tissue lesions of BF was scored 0 - 4 on the basis of lymphoid tissue necrosis and / or depletion according to Sharma et al. (1989) , the thymus 0 - 4 on the basis of cortical lymphoid tissue necrosis and / or cortical atrophy according to Nakamura et al. (1992) , and the GH was scored 0 - 3 on the basis of plasma cell (PC) necrosis and/or depletion according to Dohms et al. (1988).

Statistical analysis : Data were analyzed by students test after Steel and Torrie (1960) to determine the significance of difference between infected and corresponding controls .

RESULTS

Table (1) shows maternaly derived antibody (MDA) waining from the experimental chicks as detected by the AGPT , which exhibited a decreasing pattern weekly from 1 day up to 21 days of age , but were not detectable at 28 days of age . ElISA titers showed a similar pattern and were negative ($\leq$ 45) at 42 days of age.

Chickens inoculated with vvIBDV (Table 2) exhibited severe clinical signs including watery diarrhoea , dehydration and depression from day 2 to 7 PI , 90 % morbidity and 68% mortality occurred in 2 - 5 days PI . Severe haemorrhagic gross lesions typically seen with IBDV infection were observed in both dead and sacrificed birds . IBDV antigen could be demonstrated in all BF homogenates from birds that succumbed within 2-7 days PI but not in those collected from survivors 7 days PI .

After the initial acute inflammatory phase of IBDV-infection, which was characterized by significantly higher bursal indices than noninfected controls , BF indices declined in infected birds and were significantly lower than control values at 4, 5, 6 and 7 days PI (Table 3) . BF atrophy ,

Table (1): Waning of maternally derived antibody in commercial white egg-type male chickens.

Age/days	No. of Examined Seta	Serological tests			
		AGPT		ELISA	
		No. of Positive	%	Range	Mean ± SD
1	10	10	100	145-576	433 ± 80.95
7	10	10	100	195-432	324 ± 73.21
14	10	7	70	147-370	290 ± 45.17
21	10	2	20	101-290	202 ± 33.70
28	10	0	0	86-156	127 ± 22.50
35	10	0	0	48-120	88 ± 16.38
42	10	0	0	20-80	33 ± 18.56
49	10	0	0	18-50	20 ± 12.22

AGPT : Agar gel precipitation test . No. = Number
ELISA : Optical density X 1000 ≤ values (45 are negative).
SD : Stander deviation.

Table (2) : Results of experimental infection of commercial white egg-type male chickens with local field isolate of IBDV.

Age/ days	No. of infected Brids	Pattern of mortality PI (No. of birds dead at x day PI)							Mortality		Precipitinogen detection in BF.	
		1	2	3	4	5	6	7	Total No./ infected	%	Dead	Survivors
49	100	0	6	58	3	1	0	0	68/100	68	68/68	0/32

No. : Number
PI : post infection
BF : Bursa of Fabricius.

Table (3) : Bursa body weight mean index, bursal mean index and seroconversion of commercial white egg-type male chickens experimentally infected with vv IBDV local field isolate at 49 days of age.

Exam. time	Bird treatment	No. of Exam. Bird	Body wt (g) at x days PI.		Bursal wt (g) at x days PI.		Bursal index (a) mean ± at x days PI.	B: B indes (b) mean ± SD at days PI.	Seroconver-sion at x days PI (AGPT).
			Range	Means ±SD	Range	Means ±SD			
1	I	5	262-315	275 ± 6.25	1.25-1.65	1.40 ± 0.20	5.09 ± 0.65	1.04	0/5
	C	5	287-312	286 ± 7.11	1.20-1.55	1.40 ± 0.40	4.90 ± 0.33		0/5
2	I	5	280-340	315 ± 5.80	2.70-3.10	2.6 ± 0.20	8.25 ±0.41***	2.06	0/5
	C	5	288-370	325 ± 4.30	1.13-1.50	1.3 ± 0.20	4.00 ± 0.20		0/5
3	I	5	310-400	355 ± 6.80	2.65-3.80	2.70 ± 0.30	7.60 ± 0.35***	1.90	0/5
	C	5	330-420	370 ± 4.13	1.18-1.60	1.50 ± 0.10	4.05 ± 0.42		0/5
4	I	5	315-416	366 ± 5.40	0.90-1.40	1.10 ± 0.30	3.00 ± 0.27*	0.84	0/5
	C	5	350-430	390 ± 7.81	1.30-1.70	1.40 ± 0.20	3.59 ± 0.20		0/5
5	I	5	310-430	385 ± 6.14	0.70-1.50	0.90 ± 0.30	2.34 ± 0.38*	0.57	0/5
	C	5	370-460	433 ± 8.10	1.20-2.60	1.70 ± 0.20	3.93 ± 0.42		0/5
6	I	5	325-450	395 ± 12.15	0.60-1.40	0.80 ± 0.40	2.03 ± 0.53***	0.46	0/5
	C	5	388-485	440 ± 7.56	1.20-2.70	1.90 ± 0.20	4.39 ± 0.44		0/5
7	I	5	315-455	390 ± 15.80	0.40-1.20	0.60 ± 0.30	1.54 ± 0.45***	0.33	1/5
	C	5	410-480	450 ± 6.13	1.20-2.90	2.10 ± 0.40	4.67 ± 0.36		0/5

PI: Postinfection.
I: Infected.
C: Non-infected control
(a): Determined by the formula of Sharma et al., (189).
(b): Determined by the formula of Lucio and Hitchner (1979), where by bursa: body weight index (B:B index) less than 0.7 denoted bursal atrophy (Lucio an Hitchner (1980).
SD: Stander deviation.
AGPT: Agar gel precipitation test (positive number/examined number).
* Significantly different from control at P≤ 0.05.
***= Very highly significantly different from control at ≤ 0.001.

Table (4) : Histopathological lesion scores of the bursa, thymus and harderian gland of white egg-type chickens infected at 49 days of age with local flied isolate of IBDV.

Day PI	Bird treatment	No. of Bird	Mean lesion score (A)		
			Bursa (B)	Thymus (C)	Harderian gland (D)
1	I	5	0.7	0.0	0.0
	C	5	0.0	0.0	0.0
2	I	5	1.35	0.35	0.7
	C	5	0.0	0.0	0.0
3	I	5	3.70	0.70	1.0
	C	5	0.0	0.0	0.0
4	I	5	4.0	1.0	1.35
	C	5	0.0	0.0	0.0
5	I	5	4.0	1.35	2.70
	C	5	0.0	0.0	0.0
6	I	5	3.35	1.35	3.0
	C	5	0.0	0.0	0.0
7	I	5	2.20	1.0	3.0
	C	5	0.0	0.0	0.0

(A) : Average score of five birds .
(B) : Scored according to Sharma et al. (1989).
(C) : Scored according to Nakamura et al. (1992) .
(D) : Scored according to Dohms et al. (1988) .

I = infected .
C = control .
PI = post infection.
No. = number.

as indicated by bursa : body weight index less than 0.7 , was determined 5, 6 and 7 days PI (Table 3) . Only IBDV- inoculates developed precipitating antibody (1/5) detected at 7 days PI (Table 3) .

Lymphoid tissue lesions were first observed in BF. and were followed by necrotic changes in GH and thymus (Table 4 and Figs. 1&2) . On day 1 PI , the medulla of BF showed necrosis , particularly in the small lymphocytes . Extensive necrosis of medullary lymphoid cells were observed in BF on day 2 and 3 PI . By day 4 and 5 PI, the lymphoid follicles were cavitated with marked lymphoid cell necrosis in both cortex and medulla . By day 6 and 7 PI, BF were replaced by hyperplastic bursal epithelium and degenerative medullary cysts . The GH from IBDV- infected chickens showed slight depletion in plasma cells (PCs) at 2, 3 and 4 days PI . Compared with controls , there was marked necrosis and extensive depletion of PCs at 5,6, and 7 days PI (Table 4) and Figs. (3,4) .

Thymic lesions were detected 2 days PI (Table 4) . At 3 and 4 days PI lymphocyte depletion was prominent in some areas of the cortex . At 5,6 days PI, lymphocyte depletion had markedly progressed in the whole cortex. Atrophy of the cortex

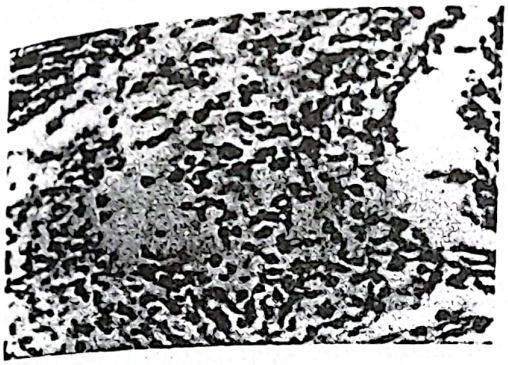

Fig.(1) : Bursa of Fabricius of chicken 4-days PI, showing edema and severe heterophilic infiltration. (H&E X 400).

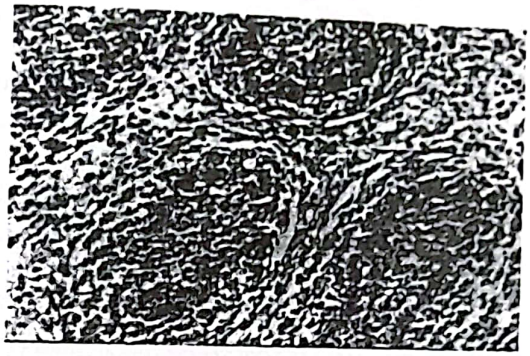

Fig.(2) : Bursa of Fabricius of chicken 5-days PI, showing degeneration of lymphoid follicles (H&E X 250).

Fig.(3) : Harderian gland of chicken 7-days PI , showing severe haemerrhage (H&E X 250).

Fig.(4) : Harderian gland of chicken 7-days PI , showing excessive lymphocytic proliferation (H&E X 250).

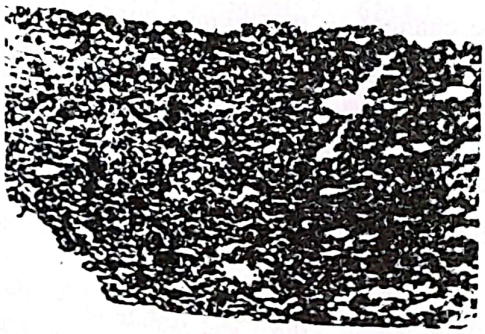

Fig.(5) : Thymus of chicken 6-days PI , showing severe haemorrhage and congestion (H&E X 100).

Fig.(6): Thymus of chicken 3-days PI , showing pronounced degeneration and depletion of lymphocytes. (H&E X 250).

was greatest at 7 days PI. In the medulla , plasma cell infiltration and microscopic haemorrhages were prominent from 5-7 days PI (Figs. 5, 6) .

DISCUSSION

Five parameters were used to evaluate the pathogenicity of an IBDV isolate for susceptible white egg-type chickens . These included clinical signs and course of the disease , morbidity and mortality rates , gross and microscopic lesions in BF, GH ,and thymus lymphoid tissues and B : B ratio and index . Based on these criteria , data indicated that the IBDV isolate is of the very virulent (vv) pathotype . Susceptible chickens inoculated with this isolate at 49 days of age exhibited severe clinical signs, gross and microscopic lesions in BF , GH and thymus and high mortality (68 %) typically seen with vvIBDV infection (Chettle et al., 1989 ; El-Batrawy, 1990 ; Inoue et al., 1994 ; Khafagy et al., 1991 ; Nunoya et al., 1992 ; Sultan ,1995) . Bursal atrophy, as judged by BF : body weight index less than 0.7 (Lucio and Hitchner , 1979 , 1980), was determined 5,6,and 7 days PI and IBDV antigen was detected in the BF of birds that died 2-5 days but not in survivors at 7 days PI . Specific serum precipitins could be detected in only 1/5 birds 7 days PI .

Histopathological investigations showed the presence of lesions in the lymphoid tissues in BF, GH, and thymus . However , the most severe lesions consisted of degeneration , necrosis and depletion of lymphocytes which were seen in the bursal lymphoid follicles . This result completely accords with the findings of Nieper et al. (1999) . Depletion of lymphoid cells in the BF by IBDV infection has to be seen in a new light since apoptosis has been observed besides necrosis in vitro (Vasconcelos and Lam, 1994; Tham and Moon, 1996) and in vivo (Inoue et al ., 1994 ; Vasconcelos and Lam, 1995; Lam, 1997 ; Ojeda et al ., 1997) . The changes in the thymus and GH were less extensive than in the bursa . This might be explained by the possible relationship between virulence and immunosuppressive effect of IBDV (Rojs and Cerne, 1997) . Detection of histopathological changes in both BF and thymus cleared that IBDV infections affect both humoral and cellular immune response (Craft et al., 1990 ; Sharma and Lee , 1983). Since the GH is a major antibody producing site in the paraocular area, the reduction in PC number at 2 to 7 days PI might compromise the local immunity in the paraocular region and the upper respiratory tract associated with IBDV infection (Dohms et al., 1988).

The changes in the thymus during first week PI with vvIBDV were characterized by cortical lymphoid depletion and focal atrophy as previously reported by Inoue et al. (1994) ; Sharma et al . (1989) and Tanimura et al . (1995) .

In conclusion , the local isolate of vvIBDV not only caused high mortality (68 %) in susceptible egg-type chickens but also was accompained by

significant pathological changes in lymphoid tissues of BF, thymus and GH , which doubtless lead to immunosuppression .

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